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Pilot Study of a Commercialized Human Papillomavirus (HPV) Genotyping Assay: Comparison of HPV Risk Group to Cytology and Histology ▽

Philip E. Castle,[1]* Mark Sadorra,[2] Francisco Garcia,[3] E. Blair Holladay,[4] and Janet Kornegay[2]

Division of Cancer Epidemiology, National Cancer Institute, NIH, DHHS, Bethesda, Maryland[1]; Roche Molecular Systems, Alameda, California[2]; University of Arizona Health Sciences Center, Tucson, Arizona[3]; and Medical University of South Carolina, Charleston, South Carolina[4]

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We evaluated a commercialized PCR assay, Linear Array, that detects 37 human papillomavirus (HPV) genotypes, using a sample of liquid cytology specimens (n = 534). We found a strong association of an increasing level of HPV risk (HPV type 16 [HPV16] > HPV18 > other carcinogenic types > noncarcinogenic types > negative specimens) with increasing severities of cytologic interpretations (P_{Trend} < 0.0005) and histologic diagnoses (P_{Trend} < 0.0005).

Carcinogenic human papillomavirus (HPV) testing has now been approved in the United States as an adjunct to cytology for triage of equivocal cytology at all ages and for general screening for women of ≥30 years old (15). Several studies have now shown that detection of specific carcinogenic HPV types, especially HPV type 16 (HPV16) and HPV18, may be useful in differentiating carcinogenic HPV-positive women at greater and lower risk of having or developing precancer, cervical intraepithelial neoplasia grade 3 (CIN3), or cancer (CIN3+) (1, 2, 8). Identifying women with persistent carcinogenic HPV infection may also be clinically useful (9). Together, these data support a role for HPV genotyping in cervical cancer screening.

Commercial HPV genotyping assays are currently under development. We evaluated one assay, Linear Array (LA; Roche Molecular Systems, Alameda, CA), a PCR-based genotyping assay that detects 37 HPV genotypes which is a commercialized version of the line blot assay (13).

MATERIALS AND METHODS

Cervical specimens and data. We acquired blinded residual PreservCyt specimens, after cytologic interpretation had been rendered, from 125 women with normal cytology, 125 women with atypical squamous cell (ASC) cytology, 125 women with low-grade squamous intraepithelial lesion (LSIL) cytology, and 165 women with high-grade squamous intraepithelial lesion (HSIL) cytology or worse (≥HSIL) from the Medical University of South Carolina. The institutional review board (IRB) of the Medical University of South Carolina approved the study, and the use of these specimens was deemed exempt from review by the NCI IRB. We subsequently excluded 12 specimens called ≥HSIL because of conditions unrelated to cervical abnormalities (e.g., endometrial carcinoma histopathology).

In addition to the original histologic diagnosis, each case underwent a pathology review to ascertain the histologic diagnosis. Importantly, six cases originally called CIN2-3 and one case called CIN3 were reclassified as CIN2, and eight cases originally called CIN2-3 and one case called CIN2 were reclassified as CIN3. Also, two cases called CIN2 and two cases called CIN1-2 were reclassified as CIN1. We were not able to retrieve histology results for four women.

To supplement the number of specimens from women with severe disease, the University of Arizona supplied 12 blinded specimens from women attending colposcopy, 10 of whom were diagnosed with CIN3 (including carcinoma in situ) and 2 of whom were diagnosed with cancer. Specimens were collected under an IRB-approved protocol, and their use was deemed exempt from review by the NCI IRB.

HPV testing. Aliquots were tested using the commercially available LA HPV genotyping test according to the manufacturer's instructions. Briefly, DNAs were extracted from clinical specimen aliquots by using a QIAamp MinElute Media kit (QIAGEN, Inc., Valencia, CA), and target DNAs were amplified by PCR. LA utilizes the PGMY09/11 L1 consensus primer system and includes coamplification of a human cellular target, β-globin (6), as an internal control. Detection and HPV genotyping are achieved by using a reverse line blot method (7, 12), and the test includes probes to genotype 37 anogenital HPV types (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51 to 59, 61, 62, 64, 66 to 73, 81, 82 subtype IS39, 82 subtype W13b, 83, 84, and 89). The only deviation from the LA product insert protocol was to implement automated sample preparation for extraction of up to 96 specimens at a time on a QIAGEN MDx platform (using a MinElute Media MDx kit according to the manufacturer's instructions) rather than processing 24 specimens per batch by the manual vacuum method. Results for six specimens were excluded because of a failure to amplify the β-globin internal control, indicating poor DNA recovery and an invalid sample.

Some women (n = 86) had also been tested previously by Hybrid Capture 2 (HC2; Digene Corporation, Gaithersburg, MD), a DNA test that targets 13 carcinogenic HPV types (HPV16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, and -68). This assay has been demonstrated to cross-react with HPV66 (3, 13), an HPV type that was recently recognized as carcinogenic (5). These 14 types were considered the carcinogenic HPV types for this study.

Statistics. HPV testing results were categorized hierarchically according to cancer risk (HPV risk category) (HPV16 > HPV18 > other carcinogenic HPV types > noncarcinogenic HPV types > PCR-negative samples). We tested the association of HPV risk groups with the severities of cytologic interpretation (normal < ASC < LSIL < ≥HSIL) (n = 522) and histologic diagnosis (normal [which included women with no biopsy taken based on a colposcopic impression of normality] < CIN1 < CIN2 < CIN3 < cancer) (n = 530), using a Pearson χ^2 test and the Mantel-Haenzel extension test for trend. We compared the agreement for detection of carcinogenic HPV by LA and HC2 by calculating kappa statistics and crude agreement with 95% confidence intervals (95% CI) and tested for statistical differences in test positivity using McNemar's χ^2 test.

RESULTS

We found a strong trend of increasing severity of cytologic interpretation with increasing likelihoods of testing positive by LA for any HPV type (P_{Trend} < 0.0005) and of testing positive

* Corresponding author. Mailing address: Division of Cancer Epidemiology and Genetics, National Cancer Institute, 6120 Executive Blvd., Room 5123, EPS MSC 7234, Bethesda, MD 20892-7234. Phone: (301) 435-3976. Fax: (301) 402-0916. E-mail: castlep@mail.nih.gov.
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TABLE 1. Relationship of HPV risk group and severity of cytologic interpretation

HPV risk group	No. (%) of specimens							
	Negative	HPV positive			Carcinogenic HPV positive			Total
		Any	Noncarcinogenic	Carcinogenic	Types other than HPV16 and HPV18	HPV18	HPV16	
Negative	101 (81)	28 (19)	8 (6)	15 (12)	10 (8)	3 (2)	2 (2)	124
ASC	48 (38)	77 (62)	14 (11)	63 (50)	45 (36)	2 (2)	16 (13)	125
LSIL	2 (2)	120 (98)	23 (19)	97 (80)	72 (59)	4 (3)	21 (17)	122
≥HSIL	9 (6)	142 (94)	7 (5)	135 (89)	51 (34)	12 (8)	72 (48)	151
Total	160	362	52	310	178	21	111	522

for a carcinogenic HPV type ($P_{\mathrm{Trend}} < 0.0005$) (Table 1). Increasing severity of cytologic interpretation was associated with more-carcinogenic HPV risk categories ($P_{\mathrm{Trend}} < 0.0005$). Among women with ASC, 38% tested negative, 11% tested positive for noncarcinogenic HPV types, 36% tested positive for other carcinogenic HPV types, 2% tested positive for HPV18, and 13% tested positive for HPV16. In comparison, among women with ≥HSIL, 6% tested negative, 5% tested positive for noncarcinogenic HPV types, 34% tested positive for other carcinogenic HPV types, 8% tested positive for HPV18, and 48% tested positive for HPV16.

Likewise, we found a strong trend of increasing severity of histologic diagnosis with increasing likelihoods of testing positive for any HPV type ($P_{\mathrm{Trend}} < 0.0005$) and of testing positive for a carcinogenic HPV type by LA ($P_{\mathrm{Trend}} < 0.0005$) (Table 2). Increasing severity of histologic diagnosis was associated with more-carcinogenic HPV risk categories ($P_{\mathrm{Trend}} < 0.0005$). Among women with CIN1, 7% tested negative, 20% tested positive for noncarcinogenic HPV types, 45% tested positive for other carcinogenic HPV types, 3% tested positive for HPV18, and 25% tested positive for HPV16. In comparison, among women with CIN3, 4% tested negative, 0% tested positive for noncarcinogenic HPV types, 23% tested positive for other carcinogenic HPV types, 9% tested positive for HPV18, and 64% tested positive for HPV16.

For a subset of 86 women, we had test results for HC2 to compare with the LA results. The kappa value between Hybrid Capture 2 and LA for detection of carcinogenic HPV was 0.74 (95% CI, 0.60 to 0.89), and the percent agreement was 88% (95% CI, 80% to 94%). There were five women who tested positive by HC2 and negative by LA for carcinogenic HPV and an equal number of HC2-negative, LA-positive results, and thus there was no statistical difference in the number of positive tests ($P = 1.0$). We also compared the detection of individual HPV types by LA and the LA results for HPV risk groups to the HC2 results (Table 3). A high percentage of infections by any HPV type detected by LA tested positive by HC2, which targets only 13 carcinogenic types but is cross-reactive with another newly designated carcinogenic HPV type (HPV66) (3, 5) and some noncarcinogenic (untargeted) HPV types (3). However, when we categorized HPV infections detected by LA according to cancer risk, we found that most of the untargeted types tested positive because there was a coinfection with one or more targeted (carcinogenic) HPV types. Among the 10 single-type HPV infections by untargeted HPV types (excluding HPV66), only 3 tested positive by HC2. In those three cases, LA detected HPV42, HPV53, and HPV67, the last two of which have been reported previously as potential cross-reactive types (3). By comparison, 19 of 26 (73%) women with single-type HPV infections by HPV66, as detected by LA, tested positive by HC2.

DISCUSSION

We found that genotyping using LA demonstrated the expected associations of HPV risk categories with severities of cytologic interpretation and histologic diagnosis. A 12% prevalence of carcinogenic HPV in women is fairly typical compared to a worldwide series (4) and is consistent with that for other U.S. populations (14). Virtually all cases of CIN3 and all five cases of cancer were carcinogenic HPV positive, with roughly 60% of all cases of CIN3+ testing positive for HPV16, as expected (11). We also noted an absence of HPV18 in CIN3, but two of five (40%) cancers were HPV18 positive, a pattern

TABLE 2. Relationship of HPV risk group and severity of histologic diagnosis

Diagnosis	No. (%) of specimens							
	Negative	HPV positive			Carcinogenic HPV positive			Total
		Any	Noncarcinogenic	Carcinogenic	Types other than HPV16 and HPV18	HPV18	HPV16	
<CIN1 or no biopsy	148 (44)	192 (56)	31 (9)	161 (47)	110 (32)	11 (3)	40 (12)	340
CIN1	6 (7)	81 (93)	17 (20)	64 (74)	39 (45)	3 (3)	22 (25)	87
CIN2	3 (6)	47 (94)	3 (6)	44 (88)	19 (38)	3 (6)	22 (44)	50
CIN3	2 (4)	46 (96)	0 (0)	46 (96)	12 (23)	5 (9)	34 (64)	48
Cancer	0 (0)	5 (100)	0 (0)	5 (100)	0 (0)	2 (40)	3 (60)	5
Total	159	371	51	320	180	22	118	530

TABLE 3. Comparison of HPV type and HPV risk group to Hybrid Capture 2 (HC2) test results for 86 women

HPV type or risk group[a]	No. of positive specimens		% Agreement[e]
	LA	HC2	
HPV types[b]			
HPV16	18	17	94
HPV18	4	4	100
HPV31	10	9	90
HPV33	3	2	67
HPV35	0	0	NA
HPV39	5	5	100
HPV45	4	4	100
HPV51	8	8	100
HPV52	5	4	80
HPV56	6	5	83
HPV58	3	3	100
HPV59	7	6	86
HPV68	2	1	50
HPV66[c]	6	6	100
HPV6	4	4	100
HPV11	0	0	NA
HPV26	0	0	NA
HPV40	1	1	100
HPV42	4	4	100
HPV44	0	0	NA
HPV53	7	6	86
HPV54	4	3	75
HPV55	4	2	50
HPV61	5	3	60
HPV62	11	9	82
HPV64	0	0	NA
HPV67	4	2	50
HPV69	0	0	NA
HPV70	3	3	100
HPV71	0	0	NA
HPV72	0	0	NA
HPV73	5	5	100
HPV81	3	3	100
HPV82	2	2	100
HPV82v	0	0	NA
HPV83	3	2	67
HPV84	5	4	80
HPV89	8	7	88
HPV risk groups[d]			
HPV16	18	17	94
HPV18	2	2	100
Carcinogenic types other than HPV16 and HPV18	36	32	89
Noncarcinogenic	10	3	30
Negative	20	2	10

[a] HPV types in bold indicate the types targeted by HC2.
[b] Includes single- and multiple-type infections.
[c] HPV66 is a newly designated carcinogenic HPV type that is not targeted but is detected by HC2.
[d] HPV risk groups are defined hierarchically according to cancer risk, as described in Materials and Methods.
[e] NA, not applicable.

that has been observed previously (10). The identification of women with carcinogenic HPV was in good agreement with the results of HC2, a pooled-probe test for carcinogenic HPV types.

In this study, we implemented an automated sample prep option that increased the batch size from 24 to 96 specimens per day. Further automation, such as strip detection and interpretation, is in development and may increase the efficiency of HPV genotyping for clinical labs. LA is currently the only validated, commercially available HPV test that is manufactured under good manufacturing practices so that the reagents are standardized. LA is currently commercially available in the United States for research use only and is a CE mark in vitro diagnostic in Europe, but it is currently in clinical trials for FDA approval as an in vitro diagnostic.

We conclude that LA may be useful for HPV genotyping. Here we demonstrated the expected correlation of HPV risk groups with the severity of cervical lesions. Compared to tests that detect a pool of carcinogenic HPV types but do not distinguish which type is present, HPV genotyping will likely be better for monitoring persistent carcinogenic HPV infection, which is a prerequisite for progression to cervical precancer.

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